Exploring the regional diversity of eukaryotic phytoplankton in the English Channel by combining high-throughput approaches

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Abstract

Monitoring marine phytoplankton is essential to understanding marine ecosystems functioning, especially in productive regions like the English Channel. This study applied high-throughput sequencing (HTS) and automated pulse shape-recording flow cytometry (PSR FCM) to investigate the spatial and seasonal variability of phytoplankton diversity in French waters of the English Channel during the ECOPEL cruises in April (spring) and July (summer) 2018. Our findings revealed significant seasonal shifts in size, structure, total red fluorescence (FLR, a biomass proxy) and community composition. PSR FCM provided high-resolution size class discrimination, revealing an increase in picoeukaryote abundance and lower FLR in summer compared to spring. HTS enabled detailed taxonomic insights: in spring, picoeukaryotes (e.g. *Ostreococcus*) dominated in the Western English Channel, except in Finistère/Celtic Seas, where microphytoplankton represented the majority of reads. Nanoeukaryotes (*Phaeocystis*) dominated in the Eastern English Channel. In summer, diversity increased, with co-dominance of picoeukaryotes (*Micromonas, Bathycoccus*, *Ostreococcus*), microphytoplankton (*Chaetoceros, Leptocylindrus, Guinardia*) and nanoeucaryotes (*Teleaulax*, *Gephyrocapsa*) in the Bay of Seine. Beyond a pronounced west-east disparity, the Bay of Seine exhibited remarkable taxonomic and functional diversity, with high local contribution to beta diversity (LCBD) values in both seasons. Diversity patterns were strongly influenced by temperature and nutrient concentrations (phosphate, nitrogen), with secondary influences from salinity and turbidity. PSR FCM further revealed sub-mesoscale variability in abundance and size structure, complementing the mesoscale patterns observed through HTS. This study highlights the importance of integrating both methods to capture fine-scale phytoplankton dynamics and high-resolution diversity, thereby enhancing ecosystem management, espcecially in nutrient-sensitive, productive marine regions.

Introduction

Phytoplankton are responsible for most of the primary production in the ocean. They contribute to the recycling of organic matter by bacterioplankton and facilitate its incorporation into microbial food webs through the microbial loop (Falkowski et al. 2012). Their abundance, composition, functional traits and spatial distribution are influenced by their physical and biogeochemical environment (temperature, salinity, nutrients availability, physical constraints; Edwards et al., 2013; Nock et al., 2016; Di Pane et al., 2022) and by their biotic interactions. Phytoplankton constitute a large polyphyletic group, with their presence in five of the eight eukaryotic "super-groups" (Simon et al. 2009; Burki et al 2020). Within these groups, different size classes can be distinguished, with varying effects on the ecology and biogeochemistry of the oceans. The English Channel (EC) is a marginal sea between the Atlantic Ocean and Celtic Seas and the North Sea and plays an essential hydrological role. The particularity of the EC lies in its complex hydrodynamic regime, characterized by strong tides and currents (Salomon & Breton, 1993), which play an essential role in shaping its oceanographic and ecological dynamics. In the Western English Channel (WEC), Atlantic Ocean waters

enter the system and flow through central offshore waters towards the North Sea. In addition, the Eastern English Channel (EEC) is subject to significant freshwater inflows to coastal waters, especially in coastal French waters, mainly from the Seine River (Dauvin, 2012) and smaller Central and Northeastern estuaries. Microphytoplankton eukaryotes, often dominated by diatoms, generally represent the largest part of total phytoplankton biomass in coastal and shelf systems (Leblanc et al., 2012). This compartment has historically been the most studied, mainly through microscopic counts, while total phytoplankton biomass was estimated by chlorophyll *a* concentration. However, in some regions, such as the Eastern English Channel, nanophytoplankton can account for over 80 % of phytoplankton biomass, with significant annual *Phaeocystis globosa* blooms (Breton et al., 2000, 2022; Seuront et al., 2006; Grattepanche et al., 2011; Lefebvre et al., 2011; Hernández-Fariñas et al., 2014; Genitsaris et al., 2015; Genitsaris et al., 2016). Moreover, eukaryotic picophytoplankton and pico-cyanobacteria play a major role during seasonal transitions in the English Channel (Tarran & Bruun, 2015; Bonato et al., 2016), dominating both photosynthetic biomass and primary production in nutrient-poor waters such as the open ocean and oligotrophic waters (McQuatters-Gollop et al. 2024). However, this latter group has often been underestimated due to their small size, which makes it impossible to count, identify and conserve using traditional methods. While picophytoplankton can be quantified using optical techniques such as epifluorescence microscopy and conventional flow cytometry, these methods typically include nanophytoplankton but exclude microphytoplankton, which must be analyzed using inverted microscopy. To obtain a complete and unbiased assessment of phytoplankton on a single approach and without the possible biases of the use of fixatives, automated pulse shape-recording flow cytometry provides a powerful alternative, enabling the characterization and count of the entire phytoplankton community across a broad size range (0.1 to 800 µm-width). This method is based on the recording of morpho-physiological characteristics such as size, pigment content and physiological state (Dubelaar et al., 1999; Dubelaar & Jonker, 2000; Haraguchi et al., 2017; Fragoso et al., 2019) derived from optical features, making it robust enough for addressing a wide range of variables, such as phytoplankton abundance, size, biomass, and processes as cell cycles. However, this quantitative method is ataxonomic and can only be used to define phytoplankton functional groups (PFGs). In order to fill this gap, high-throughput sequencing of eukaryotes enables finer taxonomic resolution of all phytoplankton size classes, as well as detection of rare species (Nolte et al. 2010). This sequencing is mainly based on the use of 18S ribosomal DNA (rDNA) markers for eukaryotes and 16S for Bacteria. Stern et al. (2023) have demonstrated the benefits of combining HTS and traditional benchtop FCM during a time series measurements in the Western English Channel, as well as for the study of harmful algae. Therefore, combining PSR FCM with HTS should enable to address, for the first time at least in the English Channel, the whole size range of phytoplankton on both their high spatio/temporal and taxonomical resolution. This study aims to investigate the spatial distribution, composition and diversity of marine phytoplankton in the sub-surface waters of the French waters of English Channel during spring and summer, considering both taxonomic composition and functional diversity across meso- and sub-mesoscales. The second objective is to assess the possible environmental and biogeochemical drivers (e.g., temperature, nutrients) influencing these distributions. Finally, the study aims to evaluate the benefit of integrating high-throughput sequencing and pulse shape-recording flow cytometry methods to characterize phytoplankton diversity and size structure more comprehensively.

Materials and methods

Sample measurements, collection and acquisition

The data in this study were collected during the ECOPEL (Pelagic Ecosystems in the English Channel) cruises, conducted in spring and summer 2018 as part of the French Marine Strategy Framework Directive dedicated cruises for the setting of the Pelagic Habitats Monitoring Programme (French Ministry of Ecology-CNRS INSU convention). These cruises conducted aboard the Antea R/V (IRD-French Oceanographic Fleet - FOF), focused on the distribution and dynamics of plankton within the French Exclusive Economy Zone (EEZ) of the English Channel and the southern North Sea (Artigas, 2018). The cruises took place in two

key periods: the onset of the *Phaeocystis globosa* spring bloom and the following summer season, period of possible harmful Algal Blooms in the Bay of Seine. Underway hydrological and PSR FCM measurements were made *in vivo* along coast-to-offshore transects within the French EEZ, from Dunkirk to Brest (Appendix 1). The two legs occurred from April 18 to May 2, 2018 (LEG1, 56 discrete samples for biogeochemical and biological HTS samples) and July 16 to July 31, 2018 (LEG2, 52 discrete samples). These campaigns were analysed and used within the framework of the Marine Strategy Framework Directive (MSFD) in a complementary way for the seasons not already explored during the optimised fishing campaigns (Baudrier, 2018; Jouandet et al., 2020).

Environmental measures and sampling

The temperature and salinity of the water column were measured using a CTD probe (SBE19, Sea-Bird Scientific, USA). In addition, temperature, salinity and turbidity were monitored continuously via the PocketFerrybox system (4H JENA engineering, GmbH), which was connected to the boat's water underway pumping system at – 2.5m-depth. On the other hand, discrete seawater samples were collected at each station from a depth of 2 meters using an 8-liter Niskin bottle. Concentrations of nitrite (NO2-), nitrate (NO3-), phosphate ($PO4^{3}$-), and silicate (Si(OH)4) were measured in duplicate surface water samples using the Aminot and Kerouel (2004) method with a Futura II autoanalyzer (AMS Alliance, Italy). Ammonium (NH4+) levels were quantified with a fluorometer (Turner Trilogy©, Turner Designs Ltd., USA) following the addition of a mixture of sodium sulfite solution, sodium tetraborate decahydrate, and orthophthaldialdehyde (Oriol et al., 2015). Dissolved and particulate organic carbon concentrations were measured using a Shimadzu TOC-L analyser (Japan), with samples filtered through GF/F 47 mm filters that were pre-decarbonated and stored in 20 mL vials acidified with 50 µL of hydrochloric acid. Particulate Organic Carbon and Nitrogen (POC and PON, in µg L^{-1}) were assessed with a NA2100 Frisons CHN analyzer. Suspended particulate matter (SPM, in mg L^{-1}) was quantified by the weight difference of pre-combusted GF/F filters (0.7 µm) before and after filtration. Chlorophyll *a* concentration (Chla, µg L^{-1}) was determined using a Turner 10-AU fluorometer (10-AU Field Fluorometer, Turner Designs Ltd., United States) and calculated according to the Lorenzen equations (1967) after acetone extraction following the Holm & Hansen (1966) method cited in Arminot and Kerouel (2004).

Pulse shape-recording automated flow cytometry analysis

Phytopankton abundance estimates were based on *in vivo* samples measured continuously underway from the ship's seawater pump and analysed directly with a pulse shape-recording automated flow cytometry (PSR FCM, CytoSense, Cytobuoy b.v., Netherlands). Unlike standard flow cytometers, this instrument is designed for analyzing the full spectrum of phytoplankton community structures, spanning sizes from 1µm to 800 µm-width at individual cells/colony level based on their pulse shape profiles (Dubelaar et al., 2004). In addition to counting cells/colonies, this technique addresses cell size (by measuring light scatter) and pigment content/presence by measuring *in vivo* fluorescence (red, orange and yellow) of each particle. These cytometry signals (Forward Scatter-FWS as a proxy of size, Sideward Scatter-SWS as a proxy of granulosity, and Red fluorescence FLR as a proxy of chlorophyll *a* in vivo fluorescence, Orange FLO and Yellow FLY Fluroscence as a proxy of Phycoerythrin-Phycocyanin *in vivo* fluorescence) enable the discrimination of phytoplankton into distinct populations, reflecting different functional groups within a sample. This distinction was made manually using cytogram analysis with CytoClus dedicated software (Cytobuoy b.v., Netherlands). Phytoplankton groups were characterised based on their optical and pigment signatures (Bonato et al., 2016, Louchart et al., 2024, Hubert et al., 2024, Robache et al., 2025). The groups were defined and named based on the standardised vocabulary defined by Thyssen et al., 2022. During the campaign, two automated cytometers were used to ensure uninterrupted sampling in the event of technical issues, as was the case in the Bay of Seine during LEG1 (this detail is documented in the submitted database, Artigas, 2018). Moreover, this approach enabled a robust intercomparison between the two instruments, allowing for reliable data completion.

Eukaryotes DNA

Subsurface seawater samples were collected at each station using Niskin bottle, pre-screened with a 150 µm mesh to retain larger particles and most metazoan, and then filtered with 0.22 µm Sterivex filters units (Millipore, Burlington, MA, USA) until clogging (volumes going from 142 mL to 6500 mL) using low filtration pressure peristaltic pump. Samples were immediately stored at -80 °C onboard until DNA extraction at the laboratory.

DNA extraction and 18S sequencing

Total nucleic acids were extracted using Qiagen AllPrep DNA/RNA Mini kit (Qiagen, Hilden, Germany) following the manufacturer's protocol, and DNA concentrations were measured with the Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Universal primers 18S-82F (5'-GAAACTGCGAATGGCTC-3', (Lopez-Garcia et al., 2003) and Euk-516r (5'-ACCAGACTTGCCCTCC-3', (Casamayor et al., 2002) were used to amplify around 480 bp of V2-V3 regions from eukaryotic 18S rDNA gene. GenoScreen (Lille, France) performed Amplicon libraries construction and Illumina MiSeq paired-end sequencing. Sequencing data was submitted to the NCBI sequence read archive database (SRA accession: PRJNA1242217).

Bioinformatics and analysis

The rDNA sequences were processed together using MOTHUR v1.47.0 software following the standard operating procedure (https://mothur.org/wiki/miseq_sop/, last access: 22 March 2025). Briefly, sequences were extracted, demultiplexed, quality filtered and aligned against the SILVA database (http://www.arb-silva.de/, last access: 22 March 2025). Suspected chimeras were removed by using UCHIME software (Edgar, 2010) and dereplicated to unique sequences. A total of 7,568,881 rDNA reads (˜66,000 reads per sample) were grouped into Operational Taxonomic Units (OTUs) at a similarity threshold of 97 %, using the mean neighbour method. Singletons, referring to OTUs represented by a single sequence within the entire dataset, were excluded, as these are typically indicative of sequencing artefacts. Finally, a total of 7,300 OTUs were taxonomically affiliated by using BLASTN against the SILVA v138 database.

Phytoplankton size range characterisation

Based on taxonomic affiliation and physiological information from both cultures and field studies, 4,944 OTUs (207,888 reads; 67.7 % of total OTUs) were classified as phytoplankton-organisms capable of photosynthesis, including autotrophs and mixotrophs, as documented in the literature (see Appendix 2). At each station, the 10 major phytoplankton genera, representing between 81.25 % and 100 % of phytoplankton reads, were manually characterised based on a literature search to assign a size class (pico-, nano-, microplankton) and a trophic mode that could indicate potential pigmentation. This analysis allowed for the investigation of the structure of the phytoplankton community and facilitated the comparison with flow cytometry data (Appendix 2). In addition, details on the capacity to form toxic or harmful algal blooms (HABs) were added in appendix 2, based on the continuously enriched list (Lundholm et al., 2025).

Diversity analyses

All analyses were performed on R software (R-project, CRAN) version 4.3.1.

Relationship between environmental parameters and phytoplankton

A heat map based on Spearman correlations allowed for the visualization of the correlations between environmental parameters. Euclidean distances were calculated for each parameter, and Mantel correlations (with 9,999 permutations) were conducted to assess relationships between environmental parameters and phytoplankton community composition determined by high-throughput sequencing and flow cytometry.

Beta diversity

The beta diversity was addressed using Hierarchical Ascendant Classification (HAC) on square root-transformed OTUs read numbers or the abundance of Phytoplankton Functional Groups, determined by automated PSR FCM. Clusters were generated based on the Bray-Curtis dissimilarity matrix (Bray and Curtis, 1957), followed by a hierarchical classification using Ward's method (Ward, 1963). Changes in the functional and taxonomic composition of eukaryotic phytoplankton communities were analyzed using the Local Contribution to Beta Diversity (LCBD, Legendre & De Cáceres, 2013; Rombouts et al., 2019; Louchart et al., 2024). This metric enables the assessment of how unique each site's community composition is in relation to overall beta diversity. A higher LCBD value indicates a site with a distinctive composition, which could reflect the presence of rare species, an unusual species assemblage, or a degraded environment with low species diversity including blooming (potentially harmful) species. Species Contribution to Beta Diversity (SCBD) was calculated in order to address the species variation between sites. A species with a high SCBD value means that it is heterogeneously distributed between sites and contributes strongly to differences in composition between them. Sites where species with high SCBD values are abundant and dominate the community typically also exhibit high LCBD indices (Legendre & De Cáceres, 2013). Both LCBD and SCBD were calculated from PFGs cell/colony and HTS reads abundance data that had been previously Hellinger-transformed to account for the high variability in species and PFGs abundance along the English Channel and between the two seasons explored. Euclidean distances were computed based on the transformed data, and calculations for LCBD and SCBD were performed using the*beta.div* function from the *adespatial* R package (Dray et al., 2022).

Results

Environmental characteristics

A comparison of the abiotic parameters recorded in sub-surface waters from the two surveys highlights their distinct seasonal characteristics (Fig. 1). As anticipated, temperatures were significantly higher in July compared to April. Spring was characterised by statistically lower salinity and elevated concentration of suspended and particulate matter, including suspended particulate matter (SPM), particulate organic carbon (POC), particulate inorganic matter (PIM), and particulate organic matter (POM). Conversely, dissolved organic carbon (DOC) concentrations were higher in summer. Nutrient analysis revealed that waters sampled in April exhibited significantly higher concentrations of nitrite, nitrate, and phosphate, whereas a peak of silicate concentrations was recorded in July. Given this marked seasonality, Euclidean clustering of environmental data was performed by season to delimit zones with similar environmental characteristics (Fig. 2). Regardless of season, a clear environmental gradient was evidenced in the Bay of Seine from the Seine river mouth to northern offshore and western bay waters. Stations near the Seine mouth consistently formed a distinct cluster (cluster 7 in both spring and summer). This cluster represented the area most directly influenced by the Seine River, characterized by the lowest salinity value, the highest turbidity and elevated concentrations of nutrients (highest Nitrate and Silicate levels and high levels of Phosphate and Nitrite in spring, highest Nitrate, Nitrite, Phosphate and Silicate levels in summer). In spring, three distinct water types were characterized in the Bay of Seine, extending northward and eastward along the coast of the Eastern English Channel (EEC) in the form of a plume of increasing salinity to the coasts of Haute-Normandy (clusters 7, 6 and 4). In contrast, four water types were observed in the Bay of Seine during summer (clusters 7, 8, 5 and 2). Further north-eastward, along the coast, the Somme and smaller estuaries formed its own distinct cluster (cluster 2 in spring and cluster 3 in summer) representing the "coastal flow" (Brylinski et al., 1991) that expanded northwards to the Strait of Dover. During summer, offshore waters of the EEC and most offshore and Gulf Normand Breton stations in the Western English Channel (WEC) were grouped into cluster 1, characterized by the lowest summer temperatures, the highest salinity, and minimal nutrient concentrations, but were more fragmented in spring (clusters 4, 5, 8). Meanwhile, the North Sea formed a separate cluster in spring (cluster 1), but these waters became more fragmented in summer (clusters

2, 3 and 4).

Phytoplankton functional diversity

During spring, eukaryotic phytoplankton abundances addressed by automated PSR FCM ranged from 818 to 18,325 cells mL-^{1}, with high values recorded off the estuarine areas (especially in the Bay of Seine; Fig. 3a) and the Strait of Dover. The highest phytoplankton abundance characterized the mouth of the Seine estuary, where nanoeukaryote (RedNano) phytoplankton were particularly abundant. Throughout the spring campaign, RedNano dominated phytoplankton abundance in most Eastern English Channel (EEC) phytoplankton communities, whereas picoeucaryotes (RedPico) were prevalent in most western (WEC) waters. RedNano was the most dominant group in terms of Red fluorescence (a proxy for chlorophyll *a* concentration and phytoplankton biomass; Fig. 3c) across English Channel waters, followed by a notable contribution of microphytoplankton (RedMicro). During the summer campaign, total eukaryote phytoplankton abundances ranged from 161 to 173,972 cells mL-^{1}, with the highest cell density observed in the Bay of Seine. Summer was marked by dominance of RedPico phytoplankton at most stations (Fig. 3b). Red fluorescence patterns revealed more diverse contributions compared to spring, with significant contributed FLR of RedNano, RedMicro, cryptophytes (OraNano) and RedPico (Fig. 3d). These observations align with chlorophyll *a* concentrations measured at each station (Appendix 3), evidenced that, regardless of the season, the highest chlorophyll *a* concentrations were measured at the Seine River mouth and off the Somme and smaller estuaries along the French coast of the Eastern English Channel, extending towards the Strait of Dover. However, concentrations were lower in summer compared to spring. Seasonal spatial clustering of phytoplankton communities defined by Phytoplankton functional Groups (PFGs) revealed differences in phytoplankton communities between English Channel regions and during the two seasons (Fig. 4). This clustering showed significant similarities with the clustering of environmental data (Fig. 2). The easternmost part of the EEC (North Sea and Strait of Dover) exhibited the highest complexity, combining several clusters in both spring (clusters 1, 2, 3, 4) and summer (clusters A, B, C, E, F). This region displayed a complex coastal-offshore and southwest-northeast gradient of RedNano (with maximum of 69.89% at coastal cluster 3 and 15% in cluster F) and RedPico (maximum of 49.64% at cluster 4 and 96.79% at cluster C). In spring, the Bay of Seine was characterized by a community comprising 55.8% of RedNano (cluster 7), together with three other additional clusters (clusters 6, 5, and 1). In contrast, the situation was more complex in summer, with no single dominant cluster characterizing the mouth of Seine estuary, even though cluster I was exclusively found in the Bay of Seine. The Bay of Seine was divided into five clusters (clusters A, D, F, H, and I) mainly composed of RedPico (79.5% cluster F to 93.1% cluster H), but also contribution of RedNano up to 15% on cluster F. In spring, clusters 9 and 10 were typical of WEC offshore waters, with the highest contributions of RedPico to the total phytoplankton abundance. Conversely, cluster 8 in spring, showing high proportion of RedPico (80.1%) was indicative of coastal to offshore waters in the western part of the WEC and near the Cotentin Peninsula. Surface waters by the Finistère tip, the Iroise Sea and the Bay of Brest were characterized by clusters 8, 10, 5, 4 of increasing proportion of RedNano (as in some coastal and offshore stations of the Bay of Seine and off Haute Normandy). In summer, clusters B and G defined coastal to offshore waters of the Gulf Normando Breton in the Western English Channel, characterized by 86 % RedPico and about 10 % RedNano, whereas off Finistère and Iroise Sea, clusters D, B G and F succeded from off shore to coastal (Bay of Brest) waters (Fig. 4).

Eukaryotic taxonomic diversity

Eukaryotic diversity expressed as relative abundance obtained from high-throughput sequencing (HTS; Fig. 5) highlighted distinct seasonal and spatial variations in community composition in French waters of the English Channel. In spring, the *Alveolata* group was highly dominant (representing about 62% of all reads) except on stations 16 and 23 in the EEC, 34 and 35 in the Bay of Seine and from Cotentin peninsula to offshore WEC waters (43 to 45). This higher taxonomic group was mainly composed by the phylum *Dinoflagellata* with a contribution of *Ciliophora* from the Bay of Seine to offshore central waters of the WEC.

Other significantly present taxonomic group included *Hacrobia* (*Haptophyta, Cryptophyta, Picozoa*) and *Stramenopiles* (*Pseudofungi* ,*Sagenista* , and *Ochrophyta*) (Fig. 5a). The Bay of Seine exhibited a group-level composition similar to that of the WEC but showed phylum-level differences, with significant representation of*Ochrophyta* and *Pseudofungi* in addition to*Chlorophyta* , *Choanoflagellida* , *Haptophyta* ,*Cryptophyta* , *Cilliophora* and *Dinoflagellata* . While*Haptophyta* represented the major phylum of *Hacrobia,* the read abundance of *Cryptophyta* and, to a lesser extent,*Picozoa* , increased around Cotentin and the WEC. In the same area,*Archaeplastida* (*Chlorophyta*) were also abundant.*Opisthokonta* (*Mesomycetozoa* , *Fungi and Cercozoa*) were detected at the tip of Finistère and Iroise Sea (stations 49 to 52). In summer, a greater and more balanced diversity was observed throughout the English Channel for the dominant groups - *Alveolata, Archaeplastida, Hacrobia,* and *Stramenopiles* (Fig. 5b). While*Alveolata* remained the dominant group at some stations like station 108, 100 or 82 where they reached up to 75%, its read abundance decreased by half compared to spring (31% of all reads). This group, primarily composed of *Dinoflagellata* and *Ciliophora* , was distributed across the entire English Channel. In contrast, the proportion of *Stramenopiles* , mainly *Ochrophyta* , increased significantly, particularly in the coastal waters of the EEC, where they accounted for up to 45% of total reads. Their relative reads abundance declined in offshore waters and in the coastal waters of Haute-Normandy, where *Alveolata* , *Archaeplastida* , and *Hacrobia* replaced them. *Stramenopiles* reached up to 25% in the coastal waters of the Bay of Seine and up to 40% in certain stations of the WEC. *Hacrobia* showed its highest relative abundance in the North Sea, in coastal stations of Haute-Normandy, in the coastal waters of the Gulf Normand-Breton, and in offshore waters of the WEC, where they accounted for up to 55% of total reads. The relative contribution of*Hacrobia* to total reads differed between the EEC, where*Cryptophyta* dominated, and the WEC, which exhibited higher diversity due to a greater proportion of *Haptophyta* , as well as contributions from *Picozoa* , *Katablepharidophyta* , and*Telonemia* . *Archaeplastida* reached its highest contribution to total reads in the waters of Normandy and the Gulf Normand-Breton.

Phytoplankton taxonomic diversity per size-class

The analysis of the ten most abundant pigmented genera per station and during the two seasons offered deeper insights into the composition and distribution of different phytoplankton size classes, defined by HTS in the EC. In spring, pico- or microphytoplankton reads dominated in most of the western English Channel, while nanophytoplankton reads were more prevalent in most of the eastern areas (Figure 6a). In terms of composition, the tip of Cotentin (stations 39 to 44), and certain stations of the Western EC (46, 48, 55) showed a significant contribution of pico-chlorophyte amongst phytoplankton diversity (ranging between 35 and 56.7 % of total reads, represented mainly by the *Ostreococcus* genus). *Micromonas* also contributed to picophytoplankton diversity (12 to 15%) in the Bay of Seine stations (35, 34, 33). From the west of the Canche estuary to the Strait of Dover, *Parmales* and *Dolichomastigaceae-B* accounted for nearly all picophytoplankton diversity. The North Sea stations were characterized by the presence of *Marine OCHrophyte-2 (MOCH-2)* . Nanophytoplankton in the eastern English Channel, especially from the Bay of Somme to the North Sea, was largely dominated by*Phaeocystis* , reaching over 85% of total reads at stations 3 and 22. In the central and western English Channel (stations 23 to 48),*Teleaulax* and *Chrysochromulina* contributed more significantly to total reads. Finistère surface waters exhibited a different composition, with the presence of dinoflagellates such as*Ansanella* , *Amphidoma* , and *Azadinium* , along with green algae from the *Chlamydomonas* genus. At the tip of Finistère, the nano-ocrophyte *Minidiscus* was notably present. The significant presence of dinoflagellates was confirmed by the dominance of microphytoplankton, in particular, the significant contribution of the genera *Biecheleria* and *Heterocapsa* at stations 49 to 57. From the Bay of Seine (station 39) to the EEC, the proportion of centric diatoms increased, with genera such as *Guinardia* ,*Eucampia* , *Ditylum* , *Chaetoceros* , *Lauderia* and*Thalassiosira* . The HTS approach indeed provides added value by detecting potential HAB-related genera even at low abundance or limited spatial occurrence, for instance, *Prorocentrum* was observed between stations 3 and 16, *Gymnodinium* at station 11,*Heterocapsa* from stations 23 to 57 (peaking at station 32),*Gonyaulax* appeared at stations 30–31 and 49–57, and*Pseudo-nitzschia* was detected sporadically at stations 20, 25, 36, and 38. In summer, the distribution shifted, with smaller phytoplankton alternating its dominance with microphytoplankton, which highest relative contribu-

tion to total reads was observed in the Bay of Seine and the Eastern English Channel, as well as at some stations of the Western English Channel (Figure 6b). The diversity of picophytoplankton at the genus level was lower (less species) than in spring and showed a more distinct distribution. *Ostreococcus* reads remained abundant in the Western English Channel (stations 101 to 106), as observed in spring, but were also abundant in the North Sea waters (stations 59, 60). *Micromonas* and *Bathycoccus* dominated the rest of the English Channel reads, with *Bathycoccus* being particularly prominent between the Bay of Seine and the Bay of Somme. Nanophytoplankton diversity was largely characterized by the strong presence of cryptophytes, particularly *Teleaulax* (from stations 77 to 99; OraNano on PSR FCM analysis), and coccolithophorids (stations 82 to 84; HsNano on PSR FCM analysis). *Chrysochromulina* also contributed to nanophytoplankton diversity at several stations. Between the Bay of Somme and the Strait of Dover (stations 62 to 71), the genus*Leptocylindrus* was highly represented, accounting for up to 68% of total reads. The Bay of Seine, along with stations 97 and 100, exhibited high proportions of the genus *Chaetoceros* . Stations 97 to 109 in the Western English Channel showed the presence of*Guinardia* (representing from 3 to 51 % at station 104). A bloom of *Prorocentrum* sp. (38% of reads) characterized station 84 off the Seine estuary, whereas the genus *Alexandrium* represented an important percentage of total reads (35 %) at station 91 offshore the Bay of Seine. Other potential HAB-forming genera such as*Dinophysis* , *Gymnodinium* , *Heterocapsa* ,*Lepidodinium* , and *Tripos* were also present. The clustering of phytoplankton communities based on OTUs appears to yield a similar spatial variability compared to that obtained by flow cytometry (Fig. 7). In spring, sub-surface waters off the Finistère (Iroise Sea) formed a distinct subset (Cluster 11), whose taxonomic composition was largely dominated by *Dinophyceae* exception was for Station 55, which exhibited a composition more similar to the rest of central and offshore waters of the Western English Channel (Cluster 10). Station 55 showed significant contributions from *Mamiellophyceae* (40.81 %),*Prymnesiophyceae* (33.47 %), and *Cryptophyceae* (10.27 %) to the total composition. Coastal waters off Cotentin Peninsula, in the central Bay of Seine and Haute Normandie, constituted a cluster (Cluster 6) characterized by near-equal contributions to total reads from*Mamiellophyceae* (32.56 %) and *Prymnesiophyceae* (31.21 %) and the highest contribution of *Cryptophyceae* to the total diversity across all clusters (21.27 %). The closest station to the Seine estuary hosted a unique assemblage (Cluster 9) with the lowest contribution of *Prymnesiophyceae* (2.54 %), the second-highest contribution of *Dinophyceae* (64.74 %), and 16.18 % of*Bacillariophyta* (e.g. *Chaetoceros, Guinardia, Ditylum, Lauderia* or *Thalassiosira*). Cluster 7 represented the Seine's area of influence, extending eastward, with 52 % *Bacillariophyta* (e.g. *Chaetoceros, Guinardia, Ditylum, Lauderia or Pseudo-Nitschia*), 10.20% *Dinophyceae* (e.g. *Levanderina, Heterocapsa* or *Biecheleria*), and 26% *Prymnesiophyceae* . Surface waters off the Bay of Veys (cluster 5), showed a diversity close to that of cluster 4, with 41.2% of *Prymnesiophyceae,* 29.6% of*Bacillariophyta* and 20.3 % of *Mamiellophyceae* . In parallel, Cluster 8 (corresponding to Bay of Seine offshore waters) exhibited a more complex composition, including 18.2 %*Trebouxiophyceae* , 31.82 % *Prymnesiophyceae* , 4.54 %*Prasino-Clade-VIII* , 13.6 % *Prasino-Clade-V* , 4.5 %*Palmophyllophyceae* , 9.1 % *Mamiellophyceae* , 4.55 %*Dinophyceae* , 4.55 % *Chloropicophyceae* , and 9.1 %*Bacillariophyta* . From the Somme estuary to the Strait of Dover, coastal waters were characterized by Clusters 3 and 4, with a high contribution of *Prymnesiophyceae* to total reads (80.39 % and 58.8 %, respectively). Stations further offshore and into the North Sea, were grouped in Cluster 2 and showed the second-highest read abundance of *Prymnesiophyceae* . The northernmost station showed a distinct composition that grouped into Cluster 1 with a mixed composition of *Bacillariophyta* , *Dinophyceae* ,*Mamiellophyceae* and *Prymnesiophyceae* . In summer, surface North Sea waters exhibited a unique taxonomic composition with Cluster 2, comprising 17.25% *Bacillariophyta (Guinardia, Chaetoceros)* , 15.29 % *Cryptophyceae (Teleaulax and Cryptomonadales)* , 43.3 % *Mamiellophyceae (Bathycoccus, Ostreococcus* and *Micromonas)* , and 17.4 % *Prymnesiophyceae (Chrysochromulina, Phaeocystis and Haptolina)* . Cluster 1 included a transition station between the Strait of Dover and the North Sea, as well as the coastal transition zone between the Bay of Seine and the Bay of Somme (Haute Normandy), along with a transect north of the Bay of Veys. Coastal waters between the Authie estuary and the Strait of Dover grouped into Cluster 3 (68 % *Bacillariophyta*), while offshore waters belonged to Cluster 4. The mouth of Seine stood out with a unique composition with station grouping into Cluster 7, which included 1.92 %*Raphidophyceae* (*Fibrocapsa*), absent in other clusters. Clusters 6 and 5, characterised by an increasing proportion of*Prymnesiophyceae* (*Chrysochromulina* and *Haptolina*) and

Dinophyceae (e.g. *Prorocentrum* , *Alexandrium* or*Gymnodinium*) and decreasing proportion of *Mamiellophyceae,* represented offshore waters of the Bay of Seine. Western English Channel surface waters consisted of three distinct clusters (Clusters 8, 9, and 10), with markedly different average compositions from the Eastern English Channel. These differences included a strong presence of*Prymnesiophyceae* (60.68 %; Cluster 10) and higher contributions of *Bacillariophyta (Chaetoceros, Thalassiosira)* and*Dinophyceae* (*Alexandrium* , *Biecheleria* ,*Dinophysis* , *Gymnodinium* , *Lepidodinium* ; Cluster 9). These three clusters showed different distributions, with some clusters such as cluster 8 grouping stations from different geographic areas, such as coastal and offshore waters of the Cotentin Peninsula, the Gulf Normando Breton, and off the Norhtern Finistère, whereas cluster 9 was only characterised in offshore WEC waters and cluster 10 by a single offshore WEC station.

Phytoplankton composition and environmental links

In spring, distance correlations and the statistical significance of Mantel's r-statistic indicated that physicochemical properties, specifically temperature and Dissolved Inorganic Phosphorus (DIP), were strongly correlated with the phytoplankton taxonomic composition (p-value < 0.001; Mantel's r ranging from 0.2 to 0.4). Additionally, Dissolved Inorganic Nitrogen (DIN) and DIP showed strong correlations with the community functional composition identified by flow cytometry (Appendix 4). In summer, the results of Mantel's test showed that turbidity was the only parameter strongly correlated with the functional phytoplankton composition (Appendix 4).

Phytoplankton diversity across English Channel and seasons

Beta diversity

The spatial phytoplankton assemblages were analyzed using LCBD (Local Contribution to Beta Diversity) values. The highest and most significant values indicated potential spatial shifts in phytoplankton composition, as determined by DNA High-Throuput Sequencing (HTS) and automated pulse shape-recording flow cytometry (PSR FCM, Fig 8). In spring, elevated and statistically significant LCBD values were observed in the Bay of Seine and at the tip of Finistère for HTS, and off the tip of Cotentin and Western English Channel (WEC) offshore waters for flow cytometry. During summer, sub-surface offshore WEC waters exhibited statistically higher LCBD values for both HTS and flow cytometry. Moreover, some stations in offshore (HTS and PSR FCM) and coastal waters of the Bay of Seine also showed elevated LCBD values. The Species Contributions to Beta Diversity (SCBD) values identified the genus (HTS) or PFGs (Phytoplankton Functional Groups; PSR FCM) that contributed most to local beta diversity. In spring, high SCBD values were observed for *Phaeocystis* (associated with RedNano),*Biecheleria* and *Heterocapsa* (both associated with RedMicro), and *Ostreococcus* (associated with RedPico) (Fig. 9). Flow cytometry data showed that RedNano and RedPico were the primary contributors to local beta diversity during this leg (Fig. 9). In summer, the SCBD highlighted *Leptocylindrus* and*Chaetoceros* (RedMicro), *Bathycoccus, Ostreococcus,* and*Micromonas* (RedPico), as well as *Teleaulax* (OraNano), as key contributors, with RedNano being the dominant marker given by flow cytometry.

Cyanobacteria diversity and abundance

In addition to eukaryotic abundances, the photosynthetic prokaryotic component was also analyzed during both spring and summer campaigns. Automated flow cytometry results showed that pico-Cyanobacteria (OraPicoProk) exhibited a distinct distribution pattern compared to other phytoplankton functional groups (Fig. 10). Abundance in summer were higher than in spring. Moreover, during both seasons, their maximum abundance was observed in the Western EC. In summer, high cyanobacterial abundance was also noted in the area between the Seine and Somme estuaries. The photosynthetic cyanobacterial community, analysed by HTS, revealed three distinct genera: *Phormidesmis ANT.LACV5.1* ;*Synechococcus_CC9902* and unidentified taxa (*NAs* , due to limitations in classification/affiliation). *Synechococcus_CC9902* dominated the dataset,

accounting for nearly all sequencing reads. However, the total number of reads was 20 times higher in summer than in spring, indicating a significant seasonal variation, in agreement with observations made through automated flow cytometry analysis

Discussion

The present study reports for the first time the combination of two high-throughput approaches, discrete (HTS) high taxonomical resolution and continuous high spatio-temporal resolution (automated PSR FCM), in the French waters of the English Channel, over two seasons.

Environmental characteristics

Water temperature showed higher variations in the Eastern than in the Western English Channel, reflecting typical seasonal patterns and differences in tidal dissipation and bathymetry, which could lead to a stratification in WEC compared to EEC (Louchart et al., 2020). This combination is known to potentially affect phytoplankton growth rates, metabolic activities and community structure (Regaudie-de-Gioux & Duarte, 2012; Dauvin et al., 2012; Mousing et al., 2014). Moreover, in addition to seasonal variability, an exceptional warming of the sea surface occurred in the English Channel during the summer of 2018 (Brown et al., 2022). The results of our study seem to indicate that the heatwave had mostly affected coastal areas and the Bay of Seine in July 2018. Salinity levels in the English Channel varied from West to East, with lower values observed off local estuaries, particularly in the EEC, indicating a freshwater influence and the presence of the Seine dilution plume and the EEC "coastal flow" (Brylinski et al., 1991; Dauvin et al., 2012). Surface salinity values of our study were consistent with normal gradients of the English Channel region (range between 26 to 35.5), though variations may occur depending on wind speed and direction as well as rainfall, potentially leading to lower salinity levels due to increased freshwater input (Kelly-Gerreyn et al., 2006; Louchart et al., 2020; Lefebvre & Devreker, 2023). The areas between these river-influenced regions (ROFIs) also exhibited distinct patterns: in the Bay of Seine, the Orne, Vire, Douve, and Seine estuaries contributed to freshwater and nutrient inputs with strong coastal gradients in both spring and summer (Cugier et al., 2005). Similarly, in the Bay of Somme and the Eastern English Channel, the "coastal flow" ROFI played a key role in shaping salinity distribution. In contrast, no strong coastal-offshore gradient was observed in the Gulf Normando-Breton (not sampled in spring because of very bad weather conditions) or in the WEC, except for the Bay of Brest. This suggests a weaker influence of freshwater inputs in these areas, likely due to distinct hydrodynamic patterns (Menesguen & Gohin, 2006). These hydrodynamic characteristics influenced the water clarity due to the presence of suspended particulate matter (SPM) but also phytoplankton cells during blooms, and nutrient concentration, which varies seasonally as stated in the EEC (Lefebvre et al., 2011). In addition, other environmental factors not accounted for in this study, such as photosynthetically active radiation (PAR), bathymetry, and the stability of the water column, may also play a role in shaping the distribution of phytoplankton communities (Louchart et al., 2020). These conditions collectively shaped phytoplankton productivity and community structure, highlighting the interplay between physical, chemical, and biological factors in the English Channel. Moreover, although these campaigns aim to capture some of the seasonal variation in phytoplankton, it is important to remind that phytoplankton can vary on timescales of just a few hours, days, or weeks, especially in a region as exposed to wind and currents as the English Channel.

Taxonomic and functional phytoplankton distribution

Phytoplankton functional groups and genera in the English Channel exhibited both spatial and seasonal variations. Additionnaly to phytoplankton abundance, the contribution of functional groups to total phytoplankton biomass can be estimated using red fluorescence acquired by flow cytometry (Haraguchi et al.,

2017). In spring, chlorophyll *a* , total abundance and *in vivo* total Red Fluorescence (FLR, from automated flow cytometry analysis) measured in sub-surface waters were highest in the EEC, particularly in areas influenced by freshwater inputs such as the Bay of Seine, the "coastal flow" (Somme, Canche, and Authie main estuaries) and the Strait of Dover, reflecting favourable conditions for phytoplankton growth. In contrast, in the WEC, lower values of chlorophyll *a* , total abundance and FLR were measured in sub-surface waters, likely due to stronger vertical stratification and lower nutrient availability. In terms of taxonomic (HTS) and functional (PSR FCM) composition, a clear distinction also emerged between the WEC, where picophytoplankton (less than 3 µm) dominated both in abundance and total reads, and the rest of the English Channel. The EEC was characterized by a higher prevalence of nanophytoplankton and microphytoplankton in the Bay of Seine in terms of reads, abundance and FLR. In summer, however, the distribution of phytoplankton size classes obtained by HTS shifted, with nanophytoplankton contributing little to taxonomic and functional diversity, while picophytoplankton and microphytoplankton alternated in dominance across different regions. In offshore waters, picophytoplankton remained the primary contributors to total taxonomic diversity, whereas in nutrient-rich coastal waters such as the Bay of Seine and the Strait of Dover, microphytoplankton (e.g., diatoms) became dominant, likely driven by local nutrient dynamics and hydrodynamic conditions. On the other hand, FCM analysis showed that picophytoplankton dominated all water types in terms of abundance in summer; however, their contribution to phytoplankton biomass (FLR) was more variable and often shared with larger size classes, including microphytoplankton (RedMicro), nanophytoplankton (RedNano), and cryptophytes (OraNano). These observations in the English Channel were associated with lower concentrations of chlorophyll *a* and FLR but higher total phytoplankton abundance. Beyond the evident seasonal effect, certain methodological biases, particularly the small volume (2 mL) analyzed by PSR FCM, may contribute to an underestimation of microphytoplankton abundance. Furthermore, while our approach to size-class discrimination in metabarcoding differs from the conventional fractionation-based methods used in other studies, it provides an indirect estimation of size-structure based on species or genus identity. However, these taxonomic proxies do not always strictly correspond to the real size-classes measured by PSR FCM, which captures *in situ* cell size distributions regardless of taxonomy (Dubelaar & Jonker, 2000; Thyssen et al., 2015). Interestingly, despite these methodological differences, the observed shifts in phytoplankton distribution and composition along environmental gradients were coherent between both approaches, highlighting the interplay between physical drivers (e.g., stratification, mixing, riverine inputs) and biological responses.

Picophytoplankton diversity

In spring 2018, the abundance of picoeukaryotes (RedPico) in the English Channel ranged from 6.8 × 10^{2} to 1.5 × 104 cells mL-^{1}. In contrast, during summer 2018, their abundance ranged from 1.6 × 10^{2} to 1.74 × 105 cells mL-^{1}, with the maximum value being more than 10 times higher than in spring in the Bay of Seine. These abundances are consistent with those reported by Not et al. (2004) and Tarran & Bruun (2015) for the WEC, where eukaryotic picophytoplankton concentrations ranged from 1 × 10^{3} to 2 × 104 cells mL-^{1} and maximum abundances were observed in May and June (early summer), ranging from 3 × 104 to 8 × 104 cells mL-^{1}. In the WEC, eukaryotic picophytoplankton were known to be predominantly composed of Chlorophyta (Not et al., 2004; Masquelier et al., 2011), with three main genera present in both seasons: *Ostreococcus* ,*Micromonas* , and *Bathycoccus* . Masquelier et al. (2011) also highlighted the dominance of picophytoplankton during the summer period. Off the coast of Roscoff, these three genera were observed throughout the year (mid-2000 to mid-2001), allowing for a detailed characterization of their presence and seasonal patterns (Not et al., 2004; Romari & Vaulot, 2004; Marie et al., 2010): *Bathycoccus* predominates in February, *Micromonas* in April, and*Ostreococcus* in June and October (Marie et al., 2010). By contrast, a more recent study on metabolites in organic particulate matter found that the genus *Ostreococcus* dominated the phytoplankton community in May, especially under high-nutrient conditions, notably at station L4 in the western English Channel (Llewellyn et al., 2015). In our study, in spring, the Eastern English Channel (EEC) showed the highest pico-eucaryote abundances (Bay of Seine) that was composed of other diversified picophytoplankton genus with lower contribution to total phytoplankton diversity (usually < 25 %):*Dolichomastigaceae-B* , *Parmales env 2* , *Parmales en 3A* , *Prasino-Clade-VIII* , *Chloropicon*

or*Pycnococcus* . However, summer samples showed a lower diversity within the genera of picophytoplankton dominated by *Ostreococcus* ,*Micromonas* , and *Bathycoccus* . Including photosynthetic picocyanobacteria in the picophytoplankton diversity reveals different seasonal dynamics in both flow cytometry and HTS data. Flow cytometry showed that Cyanobacteria (OraPicoProk) had distinct distribution patterns, with peak abundances in the WEC during spring and summer, indicating favourable environmental conditions like nutrients and temperature (Napoléon et al., 2014). In summer, higher cyanobacterial concentrations were also observed between the Seine and Somme estuaries, reflecting local environmental influences. HTS identified three cyanobacterial genera: *Phormidesmis ANT.LACV5.1, Synechococcus_CC9902* , and unidentified taxa, with*Synechococcus_CC9902* dominating, suggesting it was the primary contributor to biomass. The observed 20-fold seasonal variation from spring to summer for both FCM and HTS data likely reflects environmental changes such as temperature, light, and nutrients that favour cyanobacterial growth (Napoléon et al., 2014; Bonato et al., 2016). The presence of unidentified taxa indicates limitations in current classification and calls for further research to better characterize cyanobacterial diversity.

Nanophytoplankton diversity

In spring, the Southern North Sea (SNS) and the east of the EEC were largely dominated by nanophytoplankton (RedNano, OraNano and HsNano) with abundance ranging from 105 cells ml^{-1} to 10,747 cells ml^{-1}. This size class is composed almost exclusively of RedNano and the genus *Phaeocystis* (confirmed by HTS), which is consistent with long-standing observations in the Eastern English Channel during the spring bloom of *Phaeocystis globosa* (Breton et al., 2000; Lefebvre et al. 2011; Monchy et al., 2012; Genitsaris et al., 2015; Lefebvre & Devrecker, 2023; Skouroliakou et al. 2022; 2024). *Phaeocystis globosa* can account for up to 80 % of total phytoplankton biomass in the EEC (Breton et al., 2000; Schapira et al., 2008). Previous observations using automated flow cytometry revealed that during the spring bloom of *Phaeocystis globosa* , the RedNano group corresponded mainly to this species. Moreover, its life cycle characteristics could be tracked based on clustering resolution, allowing the extraction of sub-groups within this functional group (Guiselin, 2010; Bonato et al., 2015, 2016; Louchart et al., 2024). This genus was also found in summer in smaller proportions in the English Channel, except in the Bay of Seine and its area of influence.*Phaeocystis* maximum of abundance in the WEC was previously described in spring/early summer (Widdicombe et al., 2010; Guilloux et al., 2013; Tarran & Bruun, 2015). Correspondence between NanoRed and*Phaeocystis* in the EEC is supported by additional microscopic counts not presented here (Artigas, 2018; Jouandet et al., 2020). Other haptophyte like *Chrysochromulina* or *Haptolina* were also detected, as stated by previous observations of nanoeucaryotes in the WEC during the early summer (Tarran & Bruun, 2015). Moreover, during spring, the Haute-Normandy, Bay of Seine and the WEC were characterised by an important contribution of Cryptophytes with the genus*Teleaulax* and *Falcomonas* (Seine estuary). They can also be distinguished using automated flow cytometry based on its orange fluorescence linked to phycoerythrin pigment (Li & Dickie, 2001), called OraNano according to common vocabulary (Thyssen et al., 2022).*Teleaulax* contributed largely to total reads (28 %, station 80 in Haute-Normandy coast) over all French waters of the English Channel in summer. These two genera were previously described in spring in the Bay of Veys linked with local estuarine influence (important brackish flow from two local estuaries (Douve and Vire; Bazin et al., 2014) and at the SOMLIT-Astan station (WEC) coastal waters, as one of the dominant taxa (Caracciolo et al., 2022). Cryptophytes were most abundant in late summer and autumn in the WEC, reaching maximum abundances of 1,500–2,000 cell mL^{-1} (Tarran & Bruun, 2015), which is consistent with the peak abundance of 2,529 cell mL^{-1} for OraNano counted in the present study. OraNano bloomed in the EEC during the *P. globosa* bloom (spring) and in summer (Bonato et al., 2016). Their phycoerythrin and phycocyanin pigments enable them to effectively tolerate varying light conditions (Tarran & Bruun, 2015). Additionally, Cryptophytes can acquire nutrients at low concentrations (Skouroliakou et al., 2022) and exhibit mixotrophy, consuming *Synechococcus* (Rammel et al., 2024). In summer, offshore Eastern Channel stations were marked by the presence of coccolithophorids (*Gephyrocapsa*). Flow cytometry detected HsNano primarily in the EEC during summer, especially in the Bay of Seine and along the Haute-Normandy coast. In spring, they were concentrated around the same area as well as in the offshore WEC waters. This phenomenon was previously described in the WEC, with coccolithophore blooms from June (Garcia-Soto & Pingree, 2009)

and high abundance in the EEC in early April and early July (Bonato et al., 2016). Their tolerance to high irradiance, lower nutrient needs, and ability to use organic nitrogen or phosphorus allow coccolithophores to thrive in both high-nutrient, well-mixed and low-nutrient, stratified conditions (Van Oostende et al., 2012).

Microphytoplankton diversity

Microphytoplankton is known to contribute the most to total phytoplankton carbon biomass (addressed by inverted microscopy), except during the spring *Phaeocystis* bloom in the EEC (Schapira et al., 2008), in spite of the fact that big colonies can represent an important part of total biomass. Consistent with the red fluorescence measured during both campaigns, phytoplankton biomass appears to be ten times higher in spring than in summer, twice in term of chorophyll *a* . Moreover, while in spring almost all of the red fluorescence originates from RedNano and RedMicro populations, in summer, RedPico and OraNano also contribute significantly to the total phytoplankton biomass. The abundance of RedMicro ranged from undetected to 831 cells ml^{-1} in spring with maximum abundance and red fluorescence in the Bay of Seine. In spring, the contribution of microphytoplankton to the total phytoplankton diversity and functional composition is lower in the southern North Sea and the eastern part of the EEC (the same area where the maximum amount of nanophytoplankton and, more specifically, of the *Phaeocystis* genus was observed). In the EEC, different genera of centric diatoms co-existed in our study (*Chaetoceros, Eucampia, Radial-centric-basal-Coscinodscophyceae, Guinardia, Lauderia, Leptocylindrus, and Ditylum*). Some of these species were described as transient diatom blooms, characteristic of the end of *Phaeocystis globosa* bloom, such as *Chaetoceros socialis* or *Leptocylindrus danicus* , while *Guinardia striata* , *Coscinodiscus* spp. or *Ditylum brightwellii* are more characteristic of winter diatom communities in EEC (Skouroliakou et al., 2022; 2024). This is consistent with the summer phytoplankton diversity, where the *Leptocyindrus* genus reached over 50 % in the EEC, well after the spring bloom of *Phaeocystis* . Similarly, in the EEC, the abundance of RedMicro reached its maximum at 1500 cells $mL\text{-}^{1}$ in the Somme, Authie, and Canche estuaries. In summer, in the French waters of the WEC, high read numbers of the *Guinardia* genus were observed, which is similar to previous descriptions offshore Roscoff in late/early summer (Romari & Vaulot, 2004). The Bay of Seine and certain stations off the WEC also showed a high number of*Chaetoceros* reads, and this genus has already been documented in the EEC during summer (Jouenne et al., 2007). In spring, French waters of the WEC and the Bay of Seine were characterised by a large proportion of dinoflagellates such as*Heterocapsa* and *Biechelaria* , although the diatom*Ditylum* makes a major contribution to the phytoplankton community of the Bay of Seine and *Chaetoceros* to the Bay of Veys. The Bay of Seine is known for its strong eutrophication (Passy et al., 2016) and the occurrence of diatom blooms in spring and summer (Thorel et al., 2017). These blooms are followed by frequent proliferations of dinoflagellates, some of which, like those in the *Heterocapsa* genus, can produce toxins (Napoléon et al., 2014; Belin et al., 2021). In addition to this genus, 25 other potentially harmful algal taxa were identified across the two seasonal cruises of the present study (including mainly dinoflagellates and some diatoms). In the rest of the WEC, dinoflagellates are mainly observed in summer (and autumn) (Widdicombe et al., 2010; Napoléon et al., 2013), but this was not the case in the present study. On the other hand, dinoflagellates (including heterotrophic ones) are known to be less abundant in the EEC, ranging between 0.8 x 10^3 and 40.4 x 10^3cell L^{-1}, representing only 1 to 11 % of total phytoplankton abundance (Schapira et al., 2008). Most of these two large phytoplankton groups (diatoms and dinoflagellates) were gathered together through automated flow cytometry and clustered in the RedMicro group (excepted small diatoms like *Minidiscus* or small dinoflagellates). Microscopic counts carried out in parallel and not presented here (Artigas, 2018; Jouandet et al., 2020), showed that the contribution of dinoflagellates was greatly overestimated by HTS. These discrepancies between the two approaches have already been highlighted in studies in the North Sea (Käse et al., 2020) and in estuarine areas (Abad et al., 2016), with biases introduced during DNA extraction, gene copy number or genome size in eukaryotes as probable explanations for these differences (Prokopowich et al., 2003, Lin, 2011, Martin et al., 2022).

Relations between phytoplankton composition and environmental parameters

In spring, temperature, salinity, dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphate (DIP) were identified as significant explanatory factors likely to have influenced HTS-defined genus distribution, while DIP and DIN influenced those from FCM-defined PFGs. Previous research in the region indicates that *Phaeocystis globosa* , the dominant species in EEC in spring, begins to bloom only when silicate concentrations fall below a specific threshold (Karasiewicz et al., 2018). Furthermore, this genus is well-adapted to low levels of dissolved inorganic phosphate and nitrogen, leveraging dissolved organic phosphorus and nitrogen for growth (Sanderson et al., 2008; Breton et al., 2017). Previous studies have also noted the preference of *Phaeocystis* for high salinity conditions, which are associated with minimal precipitation and lower turbidity (Karasiewicz et al., 2018). These environmental factors help explain the spatial distribution observed in spring 2018, characterized by a predominant presence of *Phaeocystis* from the Bay of Somme to the North Sea. This pattern may be influenced by the lower turbidity and lower dissolved inorganic phosphorus (DIP) availability in these areas, despite still high nitrogen inputs from 'coastal flow', which together favor *Phaeocystis* blooms. In contrast, in the Bay of Seine, the consistently high silicate inputs and strong turbidity may limit its proliferation. In summer, only turbidity appears to have significantly influenced eukaryotic PFGs' distribution in our study.

Analyses of beta diversity

Coastal ecosystems are known for their strong spatial and temporal heterogeneity, which can influence phytoplankton concentration and composition (Martin et al., 2005). This heterogeneity is closely linked to environmental variations such as temperature (Righetti et al., 2019), salinity (Muylaert et al., 2009), nutrient concentrations (Marañón et al., 2012), light availability, and turbulence (Seuront, 2005), as well as biotic interactions like competition (Pal et al., 2009), grazing, or lysis (Grattepanche et al., 2011a; Grattepanche et al., 2011b). High values of LCBD (Local Contribution to Beta Diversity) indicate a unique phytoplankton composition at certain sampling stations or measurements compared to the other sites, with a pattern that remains relatively consistent across different methods. However, some differences were observed, particularly in spring and in some offshore areas, likely due to the inherent differences between the two approaches, which target distinct aspects of diversity and operate at different spatial and either on functional or taxonomic resolutions. Louchart et al. (2024) observed that high LCBD values in spring frequently occur under conditions of low salinity and high temperature, which corresponds to the conditions observed in the Bay of Seine, for example. In these environments, the unique community composition is notably influenced by the co-dominance of RedNano III with RedPico I, RedPico II, and RedNano II PFGs in the water bodies in spring (Louchart et al., 2024). This aligns with our study results, which show that in spring, SCBD values are higher for RedNano and RedPico, highlighting their significant contribution to community differentiation. Species that play key roles in differentiating communities between sites vary between spring and summer. The SCBD (Species Contribution to Beta Diversity) depends, in part, on the abundance of species or phytoplankton functional groups (PFGs; Heino & Grönroos, 2017; Da Silva et al., 2018; Louchart et al., 2024). Automated flow cytometry provides optical traits that are closely linked to functional characteristics (Fragoso et al., 2019), allowing SCBD to be assessed at a higher frequency across the size spectrum of community-forming phytoplankton. However, functional traits do not directly influence SCBD (Heino & Grönroos, 2017; Da Silva et al., 2018) but instead affect it indirectly through species' ecological niche characteristics (Wang et al., 2024). These functional traits have a strong influence on phytoplankton community structure (Litchman et al., 2010), and probably we need to explore further FCM-defined PFGs, in particular by defining sub-groups (as carried out by Louchart et al., 2020). Future work should improve cluster resolution beyond the current five identified in the English Channel, by both manual exploration, correspondence to images taking (imaging in flow integrated to FCM, correspondence to isolated/cultivated key taxa and lifeforms, and/or through unsupervised automated classification. Linking these results with physiological data (e.g., Fast Repetition Rate fluorometry or LabSTAF - Single Turnover Active Fluorometry) could help better connect clusters to phytoplankton functional traits. The SCBD applied to HTS (high-throughput sequencing) method identified*Phaeocystis* , *Biecheleria* , *Ostreococcus* , and*Heterocapsa* as the main genera contributing to site differentiation during

the summer season, while *Leptocylindrus* ,*Bathycoccus* , *Chaetoceros* , and *Ostreococcus* showed the highest SCBD (Species Contribution to Beta Diversity) values in summer. These genera played a crucial role in the composition of phytoplankton communities, significantly contributed to the total diversity at each site but were not necessarily the most abundant. In contrast, rare species contributed less to SCBD than more common species (Heino & Grönroos, 2017; Louchart et al., 2024). This was the case for most of the potential HAB-forming taxa identified in this study. Although a range of potentially harmful algae was detected, the majority were present at low relative abundances, and no significant HAB events were observed during the two campaigns, except for *Phaeocystis* , which was well represented and thoroughly described. This may indicate that their respective blooming conditions were not met during the sampling periods, or that such events were highly localized and therefore not captured by the discrete sampling resolution. However, the SCBD and LCBD are interesting tools for improving our understanding of community ecology, bioassessment and conservation (Legendre & De Cáceres, 2013; Da Silva et al., 2018; Rombouts et al., 2019) and can be applied to both taxa and functional groups.

conclusions

By combining two high-throughput approaches, we leveraged their complementary strengths - such as high-frequency continuous measurements for fine spatial resolution, a functional trait perspective, high taxonomic resolution, the ability to detect rare or hard-to-culture organisms, and coverage of a broad size spectrum - while mitigating their respective limitations. These include PCR biases, amplification errors and the potential overestimation of certain groups (e.g., dinoflagellates) in high-throughput sequencing, low representativity due to small sample volumes, and the potential not well-defined PFGs (need for further investigation to better characterize sub-groups) of automated flow cytometry. By combining these two high-throughput methods, this integrative approach enhances our ability to infer the small-scale (high spatio-temporal resolution) distribution of phytoplankton diversity in the English Channel, both from a functional (PSR FCM) and taxonomic (HTS) approach. However, further advances, such as the deployment of autonomous filtration/extraction systems like the Environmental Sample Processor (ESP; Hendricks et al., 2023) or the Robotic Cartridge Sampling Instrument (RoCSI; Tang et al., 2020), could provide complementary insights and improve continuous, *in situ* monitoring of phytoplankton community dynamics in autonomous platforms at high frequency. The identification of areas with exceptional biodiversity and/or the presence of species potentially harmful to humans emphasizes the importance of these findings for managing coastal waters and maintaining marine water quality of the English Channel. Furthermore, identifying factors that influence these communities will shed light on the potential impacts of climate change and human activities on marine biodiversity and ecosystem functioning (including the provision of living resources) in the region. While this study focused on two seasons, extending research to autumn and winter in the same area could yield further insights into the seasonal dynamics of phytoplankton communities. Additionally, long-term monitoring of these two approaches would provide a deeper understanding of the ecosystems' evolution over time.

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Data Availability Statement

The datasets generated and analyzed during the current study are available in the [name] repository at [persistent link to datasets]. Sequencing data have been submitted to the NCBI sequence read archive database (SRA accession: PRJNA1242217). Flow cytometry and environmental data is in the process to be uploaded to the already existing SISMER repository of French Oceanographic Cruises and, precisely, to the ECOPEL 2018 cruises (ARTIGAS Luis Felipe (2018) ECOPEL 2018 cruise, RV Antea, https://doi.org/10.17600/18000443).

Author Contributions

Zéline Hubert: Conceptualization (lead); writing – original draft (lead); formal analysis (lead); writing – review and editing (equal); **Luis Felipe Artigas:** Conceptualization (supporting); Funding acquisition and project and analysis coordination (lead); Writing – original draft (supporting); Writing – review and editing (equal); **Sébastien Monchy:** Conceptualization (supporting); formal analysis (supporting); Writing – original draft (supporting); Writing – review and editing (equal); **Claire Dédécker** : Performance of *in vivo* FCM measurements (lead), treatment of FCM raw data (lead), Writing-review and editing (equal); **Luen-Luen Li:** Writing – original draft (supporting); Writing – review and editing (equal);.

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Conflict of Interests

None declared.

Ethics statement

None required.

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Tables

Figure legends

Figure 1. Boxplot of environmental parameters between spring (green) and summer (orange) 2018 ECOPEL cruises in sub-surface French waters of the English Channel.**Figure 2.** Hierarchical ascending clustering (Ward) from the Euclidean distance matrix of environmental parameters in sub-surface French waters of the English Channel. Histograms corresponded to the mean value of each cluster, represented spatially on the maps to the right. The top panel corresponds to spring and the bottom to summer 2018 ECOPEL cruises.**Figure 3.** Phytoplankton cells abundance (a, b) and Red Fluorescence (c, d) of eukaryotes phytoplankton functional groups (PFGs) during the spring (a, c) and summer 2018 ECOPEL cruises (b, d) in French waters of the English Channel.**Figure 4.** Hierarchical ascending clustering (Ward) from the Bray Curtis distance matrix of cytometric eukaryotes abundance in French sub-surface waters of the English Channel in spring (left) and summer (right) 2018 (ECOPEL cruises). Pie charts corresponded to the mean value of each cluster, represented spatially on the maps above.**Figure 5.** Relative phylum reads of total eukaryotes composition addressed by HTS during the spring (a) and summer 2018 during the ECOPEL cruises (b) in French sub-surface waters of the English Channel.**Figure 6.** Phytoplankton major genera (10 most abundant per station) in sub-surface French waters of the English Channel during the spring (a) and summer 2018 ECOPEL cruises (b) grouped by cell size fractions.**Figure 7.** Hierarchical ascending clustering (Ward) from the Bray Curtis distance matrix of taxonomic composition in French sub-surface waters of the English Channel. Pie charts corresponded to the mean value of each cluster (left) and their spatial location (right). The top panel corresponded to spring and the bottom to summer.**Figure 8.** LCBD in sub-surface waters during spring (top) and summer (bottom) 2018 ECOPEL cruises across the English Channel using HTS and PSR FCM. Red star (inside stations dots) indicates significant LCBD (p [?] 0.05).**Figure 9.** Species Contribution to Beta Diversity (SCBD) of main genera defined by PSR FCM and HTS in spring (top) and summer (bottom) during the 2018 ECOPEL cruises in French waters of the English Channel. Only groups with above the seasonal (spring or summer) SCBD average were showed. Colors dots at the right of HTS plots indicated correspondence with PFGs (dark green: RedNano, light green: RedPico, pink: RedMicro, blue: OraNano, orange: HsNano).**Figure 10.** Cells abundance of OraPicoProk phytoplankton functional groups (PFGs) and number of cyanobacteria reads during the spring (left) and summer 2018 ECOPEL cruises (right) in French waters of the English Channel.

Appendices

Appendix 1. Map of the sub-areas examined in this study along the French coast of the English Channel**Appendix 2.** Caracteristics of most common genus of phytoplankton, their phytoplankton size range, length, diameter, trophic mode (TM; H: Heterotrophic; M: Mixotrophic; P: Photosynthetic; Pa: Parasitism), dominant pigment (DP; G: Green; YB: Yellow-brown; NP: non-pigmented), phytoplankton types (Centric diatoms: CD; Pennate diatoms: PD; Green algae : GA, Dinoflagellates : D; H: Haptophyta; Cr: Cryptophyta ; R: Raphidophyceae ; Co: Coccolithophore; Cs: Crysophytes), Harmful Algal Bloom (NK: Not known, HNT: Harmful non-toxic; or HAB; Lundholm et al., 2025) and references.

Genus	Species of study
Actinocyclus	*Actinocyclus* sp.
Actinoptychus	*Actinoptychus undulatus* ; *Actinoptychus splendens*
Akashiwo	*Akashiwo sanguinea*
Alexandrium	*Alexandrium minutum ; Alexandrium hiranoi ; Alexandrium ostenfeldii ; Ale*
Amphidoma	*Amphidoma languida*
Ansanella	*Ansanella granifera*

Genus	Species of study
Azadinium	*Azadinium dexteroporum ; Azadinium spinosum ; Azadinium trinitatum*
Bathycoccus	*Bathycoccus prasinos*
Biecheleria	*Biecheleria natalensis ; Biecheleria cincta ; Biecheleria brevisulcata*
Ceratium	*Ceratium furcoides ; Ceratium* sp.
Chaetoceros	*Chaetoceros tenuissimus ; Chaetoceros affinis ; Chaetoceros* sp. ; *Chaetoceros*
Chlamydomonas	*Chlamydomonas* sp.
Chloropicon	*Chloropicon roscoffensis*
Chrysochromulina	*Chrysochromulina* sp. ; *Chrysochromulina campanulifera ; Chrysochromulina*
Crustomastigaceae-AB	*Crustomastigaceae-AB* sp.
Cryptomonadales	*Cryptomonadales XX* _sp.
Dinophysis	*Dinophysis acuminata ; Dinophysis norvegica ; Dinophysis caudata*
Ditylum	*Ditylum brightwellii*
Dolichomastigaceae-B (or *Mamiellophyceae*)	*Dolichomastigaceae-B* sp.
Eucampia	*Eucampia* sp.
Falcomonas	Falcomonas sp.
Fibrocapsa	*Fibrocapsa japonica*
Gephyrocapsa	*Gephyrocapsa* sp.
Gonyaulax	*Gonyaulax spinifera ; Gonyaulax verior*
Guinardia	*Guinardia delicatula ; Guinardia flaccida ; Guinardia striata*
Gymnodinium	*Gymnodinium* sp. ; *Gymnodinium litoralis ; Gymnodinium nolleri ; Gymnod*
Haptolina	*Haptolina* sp. *; Haptolina hirta*
Heterocapsa	*Heterocapsa pygmaea ; Heterocapsa triquetra ; Heterocapsa niei ; Heterocapsa*
Karlodinium	*Karlodinium* sp. ; *Karlodinium veneficum*
Lauderia	*Lauderia borealis*
Lepidodinium	*Lepidodinium* sp. ; *Lepidodinium chlorophorum*
Leptocylindrus	*Leptocylindrus aporus ; Leptocylindrus danicus ; Leptocylindrus minimus*
Levanderina	*Levanderina fissa*
Mamiella	*Mamiella gilva*
Mantoniella	*Mantoniella beaufortii ; Mantoniella* clade A ; *Mantoniella* clade B ; *Manton*
Micromonas	*Micromonas commode* A1 ; *Micromonas commode* A2 ; *Micromonas* clade B
Minidiscus	*Minidiscus trioculatus*
MOCH-2	*MOCH-2 XXX* sp.
Navicula	*Navicula perminuta ; Navicula phyllepta ; Navicula* sp.
Ostreococcus	*Ostreococcus tauri ; Ostreococcus* clade B ; *Ostreococcus lucimarinus*
Parmales_env_2	*Parmales env* 2 X sp.
Parmales_env_3A	*Parmales env* 3A sp.
Phaeocystis	*Phaeocystis globosa ; Phaeocystis* sp. *; Phaeocystis pouchetii ; Phaeocystis jal*
Picochlorum	*Picochlorum* sp.
Plagiogrammopsis	*Plagiogrammopsis vanheurckii*
Pleurosigma	*Pleurosigma* sp.
Polar-centric Mediophyceae	Polar-centric-*Mediophyceae* X sp.
Prasino-Clade-VIII	*Prasino*-Clade-VIII XXX sp.
Prasinoderma	*Prasinoderma* sp. ; *Prasinoderma coloniale ; Prasinoderma singularis*
Proboscia	*Proboscia* sp.
Prorocentrum	*Prorocentrum triestinum ; Prorocentrum* sp. *; Prorocentrum donghaiense ; P*
Protoceratium	*Protoceratium reticulatum*
Prymnesiophyceae Clade_B3	*Prymnesiophyceae* Clade B3 X sp.
Prymnesiophyceae Clade_B4	*Prymnesiophyceae* Clade B4 X sp.
Prymnesiophyceae Clade_D	*Prymnesiophyceae* Clade D XX sp.
Prymnesiophyceae Clade_F	*Prymnesiophyceae* Clade F XX sp.

Genus	Species of study
Prymnesium	*Prymnesium* sp. *; Prymnesium minus ; Prymnesium kappa ; Prymnesium pa*
Pseudohaptolina	*Pseudohaptolina birgeri*
Pseudo-nitzschia	*Pseudo-nitzschia granii*
Pycnococcus	*Pycnococcus provasolii*
Pyramimonadales	*Pyramimonadales* XXX sp.
Pyramimonas	*Pyramimonas* sp. ; *Pyramimonas australis ; Pyramimonas aurea ; Pyramimo*
Radial-centric-basal Coscinodiscophyceae	Radial-centric-basal-*Coscinodiscophyceae* X sp.
Scrippsiella	*Scrippsiella* sp. ; *Scrippsiella acuminata* ; *Scrippsiella precaria*
Suessiales	Suessiales XX sp.
Teleaulax	*Teleaulax acuta ; Teleaulax* sp.
Tetraselmis	*Tetraselmis* sp.
Thalassiosira	*Thalassiosira oceanica ; Thalassiosira minima ; Thalassiosira pseudonana ; T*
Tripos	*Tripos fusus; Tripos longipes ; Tripos furca*
Woloszynskia	*Woloszynskia* sp. ; *Woloszynskia halophila*

Appendix 3. Chlorophyll *a* concentration (µg L^{-1}) in (a) Spring and (b) Summer for each sampled station.

Appendix 4 . Pairwise comparison of environmental factors with taxonomic and functional composition data in (a) Spring and (b) Summer. The actual pairwise correlation coefficient (r Spearman's correlation coefficients) values are indicated in color, while the absolute PCC values are indicated using circle with bigger size representing higher absolute PCC values between the two factors. Taxon omic community and functional composition was related to each environmental factor by Mantel tests. Edge width represents the Mantel's r statistic for the corresponding distance correlation, and edge color denotes the statistical significance (P value) based on 9 999 permutations.